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No DNA Copy Number Changes in Osteochondromas: A Comparative Genomic Hybridization Study

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ABSTRACT: *Cytogenetic changes in osteochondroma samples were studied by comparative genomic hybridization and by chromosome banding. No DNA copy number changes (15 patients) or chromosomal aberrations (9 patients) were observed in any of the patients.* © *Elsevier Science Inc., 1997*

INTRODUCTION

Osteochondroma (OC) or osteocartilaginous exostosis is a benign cartilaginous tumor with a controversial pathogenesis [1–3]. About 90% of osteochondromas arise in the long bones of the limbs in the vicinity of metaphyses. Although most of the OCs are sporadic and solitary, multiple lesions are seen in 2 rare autosomal dominant disorders: hereditary multiple exostoses syndrome and Langer-Giedion syndrome, also called tricho-rhino-phalangeal syndrome type II [4]. The OCs of these syndromes are histopathologically indistinguishable from sporadic tumors, but are more prone to transform into chondrosarcomas [5].

Cytogenetic data on OC is sparse, but a limited number of OCs have a deletion in 8q [6, 7]. Patients with hereditary multiple exostoses syndrome or Langer-Giedion syndrome have constitutional chromosomal rearrangements at 8q24, a region linked to hereditary multiple exostoses [8, 9]. Linkage studies have identified 3 chromosomal locations for the hereditary multiple exostoses syndrome at 8q24.1 (*EXT1*), the pericentromeric region of chromosome 11 (*EXT2*), and at 19p11–p13 (*EXT3*) [9–12]. Loss of heterozygosity has been reported with markers linked to *EXT1* and *EXT2* in both sporadic chondrosarcomas and chondrosarcomas derived from multiple exostoses [9–13]. Thus, it has been suggested that *EXT* genes have tumor-suppressor properties or are indeed tumor-suppressor genes [12, 14].

CGH offers the possibility to detect DNA sequence copy number changes in tumors without specific probes and/or previous knowledge of the chromosomal rearrangements [15]. In the present study we have adopted CGH to detect gains and losses of DNA sequences in 15 osteochondromas. Chromosome banding analysis also was performed on 9 tumors.

MATERIAL AND METHODS

The material for CHG comprised 15 primary OC samples obtained from 15 patients treated at the Department of Orthopaedics and Traumatology, Helsinki University Central Hospital (Table 1). The maximum thickness of the cartilaginous cap was measured in perpendicular hematoxylin and eosin–stained sections. The tumor specimens, used for the DNA extraction according to standard methods, contained in general tissue from the cap and the stalk. For tumor specimens of patients 1, 2, 4–8, 12, and 13, conventional chromosome banding analysis was performed after short-term (1–21 days) culture [16].

CGH was performed using direct fluorochrome-conjugated DNAs for all samples according to the protocol described elsewhere [15, 17] with minor modifications.

The hybridizations were analyzed using an Olympus fluorescence microscope and the ISIS digital image analysis system (MetaSystems GmbH, Altlussheim, Germany) based on an integrated high-sensitivity monochrome CCD camera and automated CGH analysis software. Three-color images (red [Texas red] for the reference DNA, green [FITC] for the tumor DNA, and blue [DAPI] for the chromosome counterstain) were obtained from 12 metaphases from each sample. DNA copy number changes were confirmed using

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Received July 2, 1996; September 20, 1996.

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0165-4608/97/$17.00
PII S0165-4608(96)00332-9

Table 1 Clinical characteristics of the 15 osteochondromas analyzed by comparative genomic hybridization[a]

Sample number	Sex/age[b]	Tumor location	Tumor size	Thickness of the cartilage cap (cm)
1	M, 21	Fibula	4.6 × 4.4 × 5.7	0.4
2	M, 24	Tibia	4.0 × 2.0 × 1.0	0.2
3	M, 14	Femur	NA	0.4
4	M, 17	Tibia	5.0 × 3.0 × 3.0	0.4
5	M, 46	Hand	1.3 × 1.0 × 1.0	0.1
6	M, 15	Femur	NA	0.3
7	M, 17	Femur	1.7 × 0.7 × 0.5	0.9
8	F, 14	Humerus	8.0 × 2.5 × 2.5	0.7
9	F, 28[c]	Scapula	NA	0.5
10	M, 57[c]	Femur	12.0 × 7.5 × 8.5	2.0
11	M, 32	Femur	6.0 × 8.0 × 5.0	1.5
12	M, 19	Femur	NA	0.3
13	M, 28[c]	Femur	10.0 × 6.0 × 6.0	0.5
14	M, 31	Scapula	6.0 × 5.0 × 2.5	0.6
15	M, 37	Tibia	NA	0.5

Abbreviations: F, female; M, male.

[a] Tumors 1, 2, 4–8, 12, and 13 had normal karyotypes after conventional G-banding analysis.

[b] Age in years at diagnosis.

[c] Hereditary multiple exostoses.

a confidence interval of 99% with a 1% error probability. In each CGH experiment, negative (peripheral blood DNA from normal donors) and positive (tumor DNA with known copy number changes) controls were included and run simultaneously with the tumor samples.

RESULTS AND DISCUSSION

The present study is the first report of CGH performed on osteochondromas. None of the 15 OC samples showed any amplifications or losses of DNA sequences by CGH. These negative CGH findings are well in line with our normal results from karyotype analysis.

Earlier karyotype analyses by others have demonstrated deletions of the distal part of 8q, but only a very limited number of tumor cases have been reported so far [6, 7]. In the present study no changes were detected at 8q24. As both CGH and chromosome banding are rough techniques that reveal only genetic changes of megabases, it still is possible that 8q24 or other chromosomal areas contain loss of heterozygosity or gene mutations, such as the frameshift mutation reported by Ahn et al. [14].

This study was supported by the Sigrid Jusélius Foundation, the Clinical Research Institute of the Helsinki University Central Hospital, the Finnish Cancer Society, and the Foundation of Orthopaedics and Traumatology, all from Finland, and from the National Council of Scientific and Technological Research (CONICET) and the National University of La Plata, from Argentina.

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